RNAseq Reveals TGX1214 Effectively Modulates Key Oncogenic Pathways in Human Pancreatic Cancer Cells

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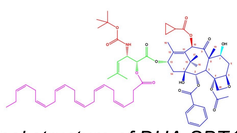
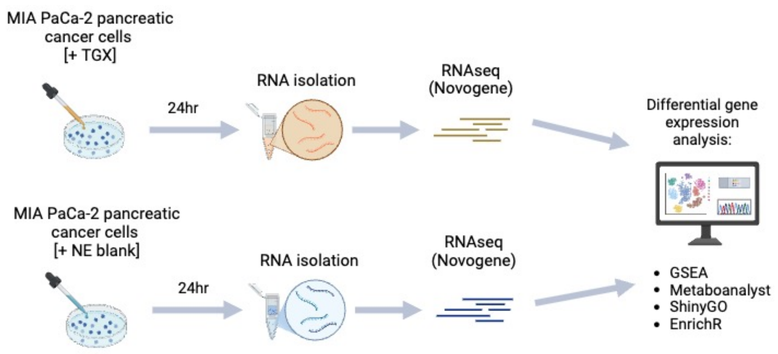
Background

- Pancreatic cancer remains one of the deadliest cancers with five-year survival rates below 10% worldwide and limited treatment options [1].
- In prior *in vitro* experiments, treatment with the novel compound TGX (DHA-SBT1214) led to significant tumor reduction and increased CD8+ T cell infiltration when used in combination with PD-L1 [2].
- The promising results of TGX have led to our current investigation of using RNAseq and comprehensive bioinformatics pipeline to study the mechanism of action of TGX
- Based on prior experiments, we hypothesize that TGX treated pancreas cells will demonstrate reduced gene expression of oncogenic pathways.



Chemical structure of DHA-SBT1214 (TGX)

Methods



- Approximately 1.2 million MIA Paca-2 cells were plated in two 6-well plates.
- For experimental wells, 12.5 nM TGX dose was administered and cells were incubated for 24 h.
- RNA isolation was performed using the Qiagen RNeasy Mini Kit (Cat. 74104) and sent to Novogene for sequencing using lumina Novaseq platform.
- Paired-end sequence reads were aligned to the GRCh38 reference genome, counted, normalized, and analyzed using the Galaxy platform
- Global gene expression profiles were examined with MetaboAnalyst, while significantly enriched pathways and key gene expression signatures were identified using GSEA, ShinyGO, and EnrichR

Results

Figure 1: Global Gene Expression Profiles

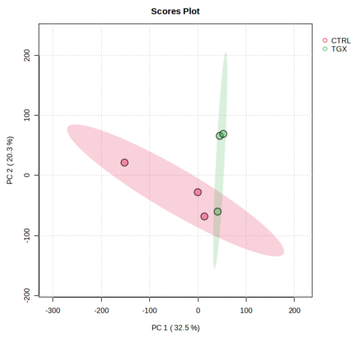
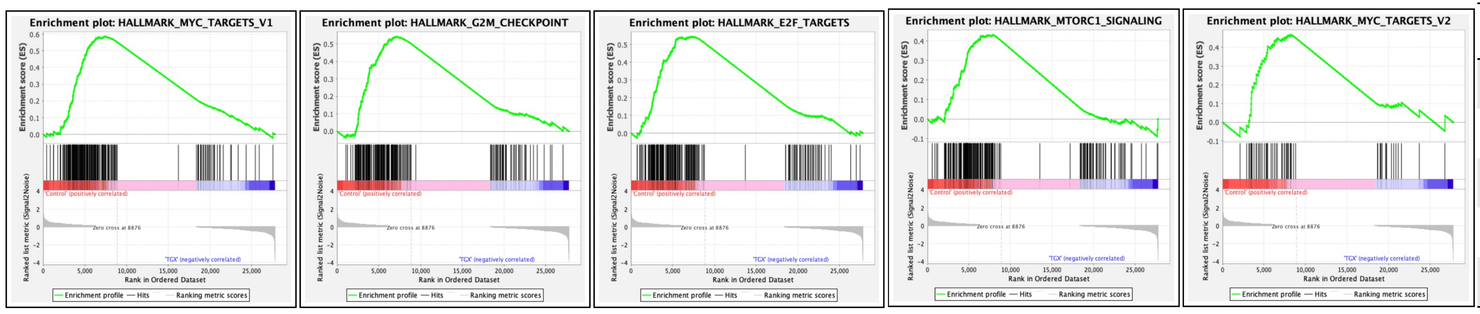


Figure 1: Principal component analysis (PCA) of gene expression profiles showing clustering of TGX-treated versus vehicle-treated pancreatic cancer cells.

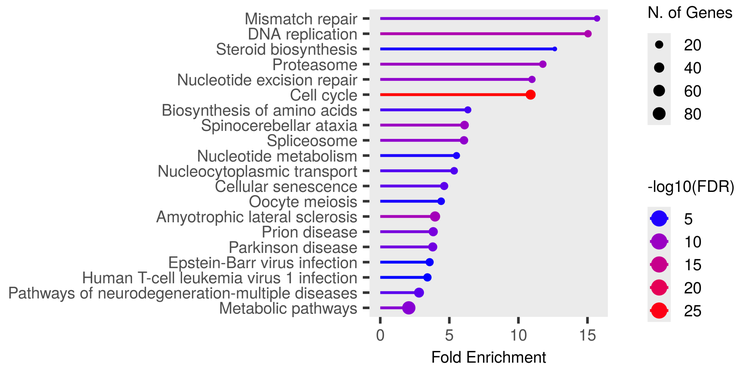
Figure 2: Five Gene Sets suppressed in TGX treated cancer cells



Gene Set	NES
MYC Targets V1	1.988
G2M checkpoint	1.86
E2F Targets	1.86
MTORC1 Signaling	1.45
MYC Targets V2	1.36

Figure 2: Gene Set Enrichment Analysis (GSEA) reveals five significantly enriched oncogenic pathways in vehicle-treated cells compared to TGX-treated cells. These pathways include MYC targets (v1 and v2), Cell Cycle Checkpoints, E2F Targets, and MTORC1 signaling. The enrichment plots show higher pathway activation in vehicle-treated cells, indicating that TGX effectively downregulates these oncogenic pathways.

Figure 3: Pathway Enrichment Analysis on Signature Genes suggests TGX abates the Cell Cycle



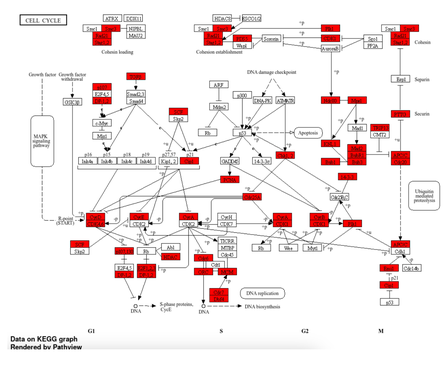


Figure 3: Bar graph depicting the fold enrichment of the top signature genes identified by GSEA, along with a cell cycle pathway map illustrating the interaction network of genes upregulated in vehicle-treated compared to TGX-treated cells. Key oncogenic pathways such as DNA replication, mismatch repair, and mitotic cell cycle control are prominently enriched in the absence of TGX treatment.

Figure 4: Identification of Perturbation Signatures Mimicking TGX treatment Effects

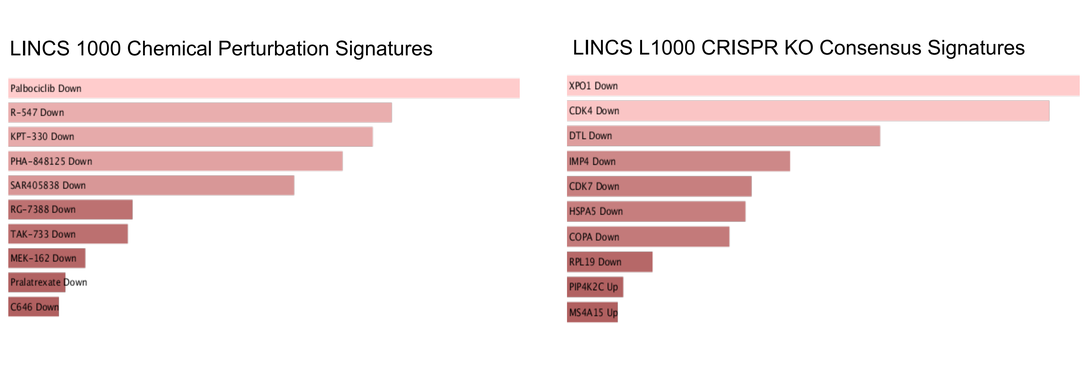


Figure 4: Analysis using the EnrichR and LINCS database identifies chemical and CRISPR perturbation signatures. CDK inhibitors, such as Palbociclib and R-547, are most likely to mimic the effects of TGX by downregulating the differentially expressed genes between vehicle and TGX-treated cells. Similarly, a CRISPR KO of XPO1 or CDK4 would result in a similar gene profile as the TGX depleted cells, indicating these proteins as potential targets of TGX.

Conclusions

- TGX treatment *in vitro* is associated with reduction of genes regulating key oncogenic pathways, including MYC, G2M cell cycle checkpoint, E2F Targets, and MTORC1 signaling.
- Reduction of tumor cell proliferation seen in prior studies may be explained by a decrease in cell cycle-related processes in TGX-treated cells, as suggested through pathway enrichment analysis.
- LINCS perturbation signature analysis implicates cell cycle proteins as potential key drivers of TGX's effect, providing potential targets for combination therapies to enhance its efficacy.
- Limitations of this data include that it is derived from a single iteration of cell culture, RNA isolation and analysis. Further replications of the experiment would be useful to validate these results.
- Next steps may also include *in vitro* validation studies with TF modification or CRISPR KO of positive or negative regulators.

Acknowledgements/ References

Acknowledgements:

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